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# STRUCTURAL MAGNETIC RESONANCE IMAGING OF BIPOLAR DISORDER

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# STRUCTURAL MAGNETIC RESONANCE IMAGING OF BIPOLAR DISORDER

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

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# ABSTRACT

Bipolar disorders are illnesses with recurring episodes of elevated or depressed mood. Although most affected individuals have periods when they are free of symptoms, they carry a life-long risk of relapse. The cause of the illness has not yet been established. During the last three decades, a growing number of brain imaging studies have shown differences in brain morphology between persons with a bipolar disorder and healthy controls, though the affected brain regions have varied greatly across studies. Too small sample sizes are likely one explanation of this variability. The aims of this thesis were to investigate i) differences in brain morphology between bipolar patients and healthy controls, ii) differences between subgroups of bipolar disorder, and iii) changes in brain morphology associated with illness progression. To these ends, we collected a large sample of bipolar disorder patients and examined their brains using magnetic resonance imaging. We then made analyses on group level.

In Study 1, we analyzed differences in gray matter volume between persons with bipolar disorder and healthy controls. We found lower volume in the bilateral insula and medial prefrontal cortex in the bipolar group. We also found that these two regions covary in size.

In Study 2, we examined if cortical volume, thickness, and surface area differ between patients with bipolar disorder I and II. We found that bipolar I subjects had thinner rostral temporal cortex than bipolar II subjects.

Many patients with bipolar disorder experience psychotic symptoms during an illness episode. In Study 3, we investigated if gray matter volume differs between patients with a history of psychosis and those without. We found lower volume in the fusiform gyrus, dorsolateral prefrontal cortex, and inferior frontal cortex in the group of patients with previous psychosis.

In Study 4, we analyzed if gray matter volume was associated with the lifetime number of manic or depressive episodes, or the duration of the illness in a group of patients with bipolar I disorder without comorbidity. We found a linear negative correlation between the volume of the dorsolateral prefrontal cortex and the lifetime number of manic episodes. This cross sectional analysis could, however, not establish if the volume reduction predates the manic episodes or is an effect of manic episodes. Therefore, we re-scanned the patients after six years. In study 5, we then compared those patients who had had at least one manic episode between baseline and follow-up with those who did not. We found that the volume in the dorsolateral and inferior frontal cortex decreased in the group who had had a manic episode.

In summary, the studies suggest that bipolar disorder is associated with reduced gray matter volume in brain areas responsible for emotional regulation. We also found that the brain morphology differs between subgroups of bipolar disorder. Finally, our results suggest that manic episodes cause gray matter volume reduction in regions coupled to cognitive functions that tend to be impaired in bipolar disorder.



## LIST OF SCIENTIFIC PAPERS

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- IV. CARL JOHAN EKMAN, Johanna Lind, Eleonore Rydén, Martin Ingvar, Mikael Landén. *Manic episodes are associated with grey matter volume reduction - a voxel-based morphometry brain analysis*. ACTA Psychiatrica Scandinavica, 2010;122:507-15
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## LIST OF ABBREVIATIONS

AC	Anterior commissure
ACC	Anterior cingulate cortex
ADE	Affective Disorder Evaluation
ADHD	Attention-deficit / Hyperactivity disorder
BD I	Bipolar disorder type I
BD II	Bipolar disorder type II
CSF	Cerebrospinal fluid
DARTEL	Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra
DSM-IV	Diagnostic and Statistical manual for Mental disorders, 4 <sup>th</sup> Edition
FWE	Family-Wise Error
GM	Gray matter
MNI	Montreal Neurological Institute
MRI	Magnetic Resonance Imaging
mPFC	Medial prefrontal cortex
SBP	St. Göran Bipolar Project
SNP	Single nucleotide polymorphism
SPM	Statistical Parametric Mapping
VBM	Voxel-Based Morphometry
WM	White matter



# 1 INTRODUCTION

This thesis rests on the underlying assumption that bipolar disorder is a brain disease and that humans with bipolar disorder demonstrate specific alterations in brain morphology that can be captured with modern brain imaging techniques. Specifically, we aimed to test the hypotheses that bipolar disorder is associated with differences in volume in certain regions of the brain, that brain morphology differs between subtypes of bipolar disorder, and that progression of the illness leads to changes in brain morphology. To these ends, more than two hundred persons with bipolar disorder and more than one hundred healthy persons have been brain scanned using magnetic resonance tomography.

Bipolar disorder is an illness of recurring episodes of abnormal states of mood. Its clinical presentation consists of pathologically elevated or severely depressed mood. During these mood episodes, the affected person's behavior is notably changed and dysfunctional. A habitually calm and timid person can become talkative, extravagant, flirty, and loud during mania. Conversely, a joyful and energetic person can become introvert, apathetic, and suicidal during depression. Bipolar disorder carries a life-long risk of relapse, even if the acute phases are successfully treated. The disease burden in terms of mental and psychical health, premature death, social and economic factors is staggering on group level. Bipolar disorder entails high societal costs, due to long periods of inpatient care and lost productivity (M. Ekman, Granstrom, Omerov, Jacob, & Landen, 2013; Peele, Xu, & Kupfer, 2003). Needless to say, the illness also causes great concern for relatives and friends. Moreover, the suicide rates in bipolar disorder have been estimated to 7.8% in men and 4.9% in women (Nordentoft, Mortensen, & Pedersen, 2011).

The causes of the illness remain unknown despite considerable research efforts in the last decades. New technology has enabled genetic studies, neurochemical analyses, as well as structural and functional examinations of the brains of living humans. But clinical studies of bipolar disorder are complicated by several factors. First, the course of the illness, with completely opposite symptoms during mania and depression, makes it difficult to separate state from trait. Second, cross-sectional studies of euthymic patients cannot separate causal factors from effects of the disease. For example, cognitive impairment has been found in persons with bipolar disorder when compared with healthy controls, but it has been difficult to establish whether this is a premorbid trait or a consequence of the disease.

The clinical presentation of bipolar disorder is heterogeneous. The cardinal behavioural symptom during a manic episode can be euphoria and expansive behaviour, but also severe irritability. Many – but not all - patients experience psychotic symptoms during an episode. Some patients are completely recovered between mood episodes whereas others suffer from lingering mood symptoms or cognitive deficits. The dominant polarity of the recurring episodes also varies. Some patients have more depressive episodes and other more manic episodes. One reason for this diversity is due to the definition of the illness: The criteria for a

bipolar I diagnosis, in the contemporary diagnostic manuals, implicate that one manic episode is sufficient for a bipolar disorder diagnosis.

The first study of brain structure in bipolar disorder was published in 1981. It was an analysis of ventricular volume, derived from computerized tomography (CT) scans. The study showed larger ventricular volumes in the bipolar sample (Pearlson & Veroff, 1981). Modern brain imaging studies, which usually have employed magnetic resonance tomography, have revealed wide-spread alterations in brain matter associated with bipolar disorder. But the findings have varied significantly across studies. This has probably been due to small sample sizes that precluded correction for medication and comorbid conditions. Different methods for processing and analyzing the images have also affected the results. Meta-analyses have attempted to reconcile these inconsistencies, but are hampered by publication bias. One meta-analysis based on raw data rather than positive results demonstrated reduced gray matter volume in the right insula in patients with bipolar disorder (Selvaraj et al., 2012). Most brain imaging investigations of bipolar disorder have been cross-sectional studies of euthymic patients. The findings can therefore be interpreted either as brain structures that render the brain susceptible to develop mania or depression, or brain alterations caused by the illness.

The aim of this thesis was to disentangle some of the inconsistencies regarding brain morphology in bipolar disorder that previous research has shown. In order to do that, efforts were made to collect a large group of persons with bipolar disorder and to perform a thorough phenotyping of each participant.

This first chapter of the thesis will give an overview of bipolar disorder, neuropathology, methods for analyzing magnetic resonance images, and the cortical brain regions that are discussed in this thesis.

## **1.1 BIPOLAR DISORDER**

### **1.1.1 History and conceptualization**

People with recurrent mania and depression have been described in medical literature from ancient Greece and Persia. The words *melancholia* and *mania* can be found in Greek medical literature from 2500 years ago. Six hundred years later, the Turkish physician Aretaeus of Cappadocia described episodes of mania and melancholia in the same person. He also distinguished melancholia in young persons from dementia, and mania from delirium. In the 16<sup>th</sup> century, it was suggested that mental illnesses were disorders of the brain and that the two states of melancholia and mania could be symptoms of one illness. In 1735, the Dutch botanical expert Hermann Boerhaave wrote: “*If melancholy increases so far, that from the great motion of liquid in the brain, the patient be thrown into a wild fury, it is called madness [mania] which differs only in degree from the sorrowful kind of melancholy, is its offspring,*

*produced from the same causes*” (Boerhaave, 1735). The bipolar illness was thoroughly described by the German psychiatrist Emil Kraepelin in 1921, in his book *Manic-depressive insanity and paranoia*. He described a heterogeneous group of patients with the common symptoms of gradually alternating mood-states, fluctuating between mania and melancholia with different frequencies and sometimes persisting and/or mixed symptoms. He hypothesized that, despite the diversity among symptoms, they were presentations of a single morbid process. This view of the illness has persisted over the years and is the fundament of today’s syndromal diagnosis Bipolar Disorder. The current definitions of bipolar disorder that is used in clinical practice and scientific research are described in the two major diagnostic manuals the Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Statistical Classification of Diseases and Related Health Problems (ICD). The ICD-10 defines bipolar disorder as:

“A disorder characterized by two or more episodes in which the patient's mood and activity levels are significantly disturbed, this disturbance consisting on some occasions of an elevation of mood and increased energy and activity (hypomania or mania) and on others of a lowering of mood and decreased energy and activity (depression). Repeated episodes of hypomania or mania only are classified as bipolar.”

Some symptoms of bipolar disorder are shared with other psychiatric syndromes. First-episode bipolar depression is very difficult to separate from a first depressive episode in a unipolar depressive illness. A manic episode with psychotic symptoms can be very hard to separate from other acute psychotic episodes in for example a paranoid schizophrenia. It is the course of the illness that is unique for the bipolar syndromes. Typically, the first episode of the illness occurs in the late teens - early twenties. Unlike schizophrenia, the first episode is not preceded by a prodromal phase of impaired function, and typically the first episode is followed by full remission.

### **1.1.2 Epidemiology**

The prevalence of bipolar disorder is about 2% of the population. A recent review has estimated the lifetime prevalence of bipolar disorder type I to 1.06% and type II to 1.57% (Clemente et al., 2015). Nationality, ethnicity, and socio-economy all have limited impact on prevalence figures. Family history of bipolar disorder is a major risk factor. Whereas the lifetime risk for the disorder is 0.5-1.5% in the general population, first degree relatives of a person with bipolar disorder have a risk of 5-10%. The concordance rate in monozygotic twins is 40-70% (Craddock & Jones, 1999). The illness also carries an increased risk for other psychiatric illnesses. Comorbid anxiety disorders, ADHD, substance misuse, and borderline personality disorder are common. It has been estimated that 67% of bipolar I patients and 76% of bipolar II have a comorbid psychiatric condition (McElroy et al., 2001).

### 1.1.3 Mania and Hypomania

The manic episode is the core clinical presentation of bipolar disorder that is unique for the illness and separates it from recurring depressive illness. During a manic episode, the mood is elevated, expansive, and/or irritable. Energy is increased, the self-esteem is inflated. The patient with mania often presents as grandiose, talkative, and agitated. The racing of thoughts, loose associations, and rapid speech sometimes make the manic patient incomprehensible and unable to uphold a normal communication. A milder form of elevated mood is called hypomania. ICD-10 defines hypomania and mania as follows:

“Hypomania: A disorder characterized by a persistent mild elevation of mood, increased energy and activity, and usually marked feelings of well-being and both physical and mental efficiency. Increased sociability, talkativeness, over-familiarity, increased sexual energy, and a decreased need for sleep are often present but not to the extent that they lead to severe disruption of work or result in social rejection. Irritability, conceit, and boorish behaviour may take the place of the more usual euphoric sociability. The disturbances of mood and behaviour are not accompanied by hallucinations or delusions.”

“Mania: Mood is elevated out of keeping with the patient's circumstances and may vary from carefree joviality to almost uncontrollable excitement. Elation is accompanied by increased energy, resulting in overactivity, pressure of speech, and a decreased need for sleep. Attention cannot be sustained, and there is often marked distractibility. Self-esteem is often inflated with grandiose ideas and overconfidence. Loss of normal social inhibitions may result in behaviour that is reckless, foolhardy, or inappropriate to the circumstances, and out of character.

#### **DSM IV-criteria for a Manic episode**

**A. A distinct period of abnormally and persistently elevated, expansive, or irritable mood**, lasting at least 1 week (or any duration if hospitalization is necessary)

**B.** During the period of mood disturbance, three (or more) of the following symptoms have persisted (4 if the mood is only irritable) and have been present to a significant degree:

- (1) **increased self-esteem or grandiosity**
- (2) **decreased need for sleep** (e.g., feels rested after only 3 hours of sleep)
- (3) **more talkative** than usual or pressure to keep talking
- (4) **flight of ideas** or subjective experience that thoughts are racing
- (5) **distractibility** (i.e., attention too easily drawn to unimportant or irrelevant external stimuli)
- (6) **increase in goal-directed activity** (either socially, at work or school, or sexually) or psychomotor agitation
- (7) **excessive involvement in pleasurable activities** that have a high potential for painful consequences (e.g., engaging in unrestrained buying sprees, sexual indiscretions, or foolish business investments)"



### 1.1.4 Depression

Depression is a very common syndrome in the general population. Neither the diagnostic criteria in the DSM-IV or the ICD-10 separate bipolar depression from unipolar depression. Nevertheless, studies have suggested that some symptoms are more common in bipolar depression than unipolar depression: psychomotor retardation, early awakening and worsening of mood in the morning, difficulty to think, and psychosis (Cuellar, Johnson, & Winters, 2005; Forty et al., 2008; Mitchell & Malhi, 2004).

#### DSM-IV Criteria for Major Depressive Disorder (MDD)

- **Depressed mood or a loss of interest or pleasure** in daily activities for more than two weeks.
- Mood represents a change from the person's baseline.
- Impaired function: social, occupational, educational.
- Specific symptoms, at least 5 of these 9, present nearly every day:
  1. **Depressed mood or irritable** most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful).
  2. Markedly **diminished interest** or pleasure in all, or almost all, activities most of the day, nearly every day
  3. Significant **weight loss** when not dieting or **weight gain** (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.
  4. **Insomnia or hypersomnia**
  5. **Psychomotor agitation or retardation**
  6. **Fatigue or loss of energy**
  7. **Feelings of worthlessness or excessive or inappropriate guilt**
  8. **Diminished ability to think or concentrate**, or more indecisiveness
  9. **Suicidality**: recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

### 1.1.5 Subtypes of bipolar disorder

In DSM-IV, Bipolar I disorder is characterized by one or more manic episode/s. Bipolar disorder type II requires one or more hypomanic episodes and one or more major depressive episodes. The ICD-10 criteria require two or more episodes also for Bipolar I.

### 1.1.6 Euthymia

Euthymia or euthymic mood define the state when a patient does not fulfill criteria for a depressive, manic, or hypomanic episode. Euthymia does not imply that the patient has

regained complete function. It may take months to recover after a mood episode. Several studies have for example shown cognitive deficits in the euthymic state (Bourne et al., 2013). The deficits are spread over different cognitive domains, e.g., executive function, verbal learning and memory, attention, and response inhibition.

### 1.1.7 Psychosis

The term *psychosis* was introduced into the medical literature by Ernst von Feuchtersleben in 1845 (Beer, 1996) and has since been used for a variety of symptoms related to mental illness. It was initially used as a synonym to *psychopathy*, separating melancholia, mania, dementia, and idiocy from *neuroses* such as cramps, palsies, and neuralgia. In the 1920's, Kraepelin defined the psychoses to only include dementia praecox and manic-depressive insanity (Beer, 1996). Throughout the twentieth century, the Kraepelinian dichotomy has been challenged by the identification of atypical psychoses and schizoaffective psychoses among others. Consequently the term psychosis has shifted from classifying syndromes to an umbrella term for a group of symptoms.

In the two diagnostic manuals, DSM-IV-TR and ICD-10, psychosis is used as a label for symptoms of perceptive, cognitive, and psychomotor dysfunction including hallucinations, delusions, disorganized speech, and disorganized or catatonic behaviour (American Psychiatric Association, 2000; World Health Organisation, 1992). Still, the definition of psychosis varies across different diagnoses even within the DSM-IV-TR. Whereas hallucinations and delusions are considered psychotic in mood disorders, only those hallucinations that are not accompanied by insight are defined as psychotic in substance use disorders or in psychosis due to a general medical condition.

In ICD-10, the severity of psychotic symptoms must be taken into account. Diagnosing a depressive episode with psychotic symptoms requires “the presence of hallucinations, delusions, psychomotor retardation, or stupor so severe that ordinary social activities are impossible” (World Health Organisation, 1992). Psychotic symptoms during a manic episode are diagnosed when “delusions or hallucinations are present, or the excitement, excessive motor activity, and flight of ideas are so extreme that the subject is incomprehensible or inaccessible to ordinary communication” (World Health Organisation, 1992).

In schizophrenia, psychosis is a *sine qua non* that defines the illness. However, lifetime prevalence of psychosis is also common in other illnesses such as bipolar disorder, substance use disorders, and temporal lobe epilepsy. Moreover, in surveys of the general population 10-25% of responders report having experienced at least one isolated psychotic symptom (Preti, Bonventre, Ledda, Petretto, & Masala, 2007) even though only a small fraction of these subjects fulfil criteria for a mental illness (Kendler, Gallagher, Abelson, & Kessler, 1996). In bipolar disorder, psychotic symptoms has been reported to occur in up to 68% of patients with a manic episode (Canuso, Bossie, Zhu, Youssef, & Dunner, 2008), in 11% of patients

with a bipolar depression, and in 25% of patients with a mixed state (Perugi, Akiskal, Micheli, Toni, & Madaro, 2001).

## **1.2 FUNCTIONAL NEUROANATOMY**

### **1.2.1 Atlases**

To describe a certain region in the brain, researchers must refer to the some atlas or other framework. There are several different atlases available. One of the oldest and possibly most well-known is the Brodmann definitions of the cerebral cortex. In 1909, Korbinian Brodmann, a German neurologist, divided the brain surface into 52 different areas based both on gross anatomical features such as gyri and sulci, and on cytoarchitecture, i.e., the organization of cell layers. A contemporary atlas was created by Talairach and Tournoux in 1988, based on dissection of one single brain. This is a three-dimensional coordinate system with the origin set to the anterior commissure (AC), an easily distinguishable bundle of nerve fibers connecting the left and right temporal lobes. An adaptation of the Talairach space, the Montreal Neurological Institute (MNI) space, is the most commonly used coordinate system in neuroimaging to date, and used throughout in this thesis. This system is derived from MRI-scans of a large number of healthy individuals that were scaled to match the Talairach brain. An average of these T1-weighted scans was used to create a template. The resulting MNI-brain is, however, slightly larger than the Talairach brain. The MNI coordinate system also originates in the AC and the line between the anterior and posterior commissure is horizontal in a sagittal image. In the axial and coronal planes, the interhemispheric fissure is vertical. The coordinates are labeled X = Left (negative numbers) to Right (positive), Y = Caudal (-) to Rostral (+), and Z= Ventral (-) to Dorsal (+).

### **1.2.2 Causes of volumetric change**

In the last three decades, the possibility to study brains of living individuals have been greatly facilitated by the development of more advanced radiological technical equipment. Magnetic resonance imaging (MRI) can now produce high-resolution images of soft tissues, and does not expose the study subjects to radiation. Neuropathological processes in brain tissue that previously only could be analyzed in post-mortem material can now be visualized and analyzed in living patients. MRI scanners are therefore frequently used in neurology and psychiatry clinics. Also, the scientific neuroimaging field is in a very expansive phase. Still, MRI does not have sufficient resolution to visualize which specific pathological processes that underlie the volume change.

### **Why would the structure and shape of the brain be altered in bipolar disorder?**

- A regional decrease in brain volume can cause impaired emotional regulation. This has mainly been documented in patients with traumatic head injury affecting the frontal lobes, but also in degenerative illness such as frontotemporal dementia.
- A regional decrease in gray matter volume has been associated with cognitive impairment. This is found for example in Alzheimer's dementia. Patients with bipolar disorder often exhibit cognitive impairment that is correlated to the duration and severity of the illness (Tham et al., 1997).
- Bipolar disorder is highly hereditary, therefore there may be neurodevelopmental aberrations associated with the illness.

### **Why would the structure and shape of the brain not be altered in bipolar disorder?**

- In most illnesses with morphological brain changes, the symptoms are constant or gradually worsening. In bipolar disorder, the symptoms are diverse and fluctuating.
- A large percentage of patients show no symptoms in-between episodes. Some may even recover completely without medication.

### **What causes reduced brain gray matter volume?**

- Trauma, hemorrhage, infarction, infection that leads to necrosis
- Apoptosis (Gigante et al., 2011)
- Less dendritic branching. In schizophrenia, post mortem studies have shown reduced dendritic density in the prefrontal cortex (Glantz & Lewis, 2000).
- Decreased cellular (neuronal) volume.
- Less myelination (Vostrikov & Uranova, 2011)
- Synaptic pruning
- Less glial cells and oligodendrocytes (Ongur, Drevets, & Price, 1998; Rajkowska, Halaris, & Selemon, 2001)

### **What confounds studies of brain morphology based on MRI-scans?**

Magnetic resonance imaging is not a photograph of the brain, neither an X-ray image. There are several factors, both physiological and technical, that can affect the MRI-signal of the water molecules, the conversion of signal to an image, and the pre-processing of images for group analyses. Thus, several factors may confound the results of MRI-scans:

- Magnetic field inhomogeneity. The magnetic field strength is not the same in the whole field of view. Stronger magnets produce a more heterogeneous field. This is

less of a problem in analyses of small parts of the brain, but for whole-brain analyses this bias must be taken into account.

- Partial volume effects. Each voxel has a volume and an intensity. The intensity of a voxel is the mean intensity of everything inside the volume of the voxel. This means that voxels on the border between tissue and water will be classified as either water or tissue. Hence, large voxels with a small proportions of high-intensity tissue can be mis-classified as tissue.
- Blood flow. One study has shown that a single dose of baclofen (anti-spastic drug) increases blood flow to a degree that gives a false positive increase in gray matter volume in Voxel-Based Morphometry-analyses (VBM) (Franklin et al., 2013).
- Brains of healthy persons are not identically shaped. Gray matter patterns and sulcal structures can differ more than one centimeter from one brain to another. Therefore can group analyses with small sample sizes be confounded by normal variation in brain structure.
- Medication. A VBM study of healthy persons taking lithium for 11 days demonstrated a gray matter volume increase whereas an analysis of the same subjects with another method did not. This difference may be caused by the effect of lithium reducing the T1 relaxation time in gray matter (Cousins, Aribisala, Nicol Ferrier, & Blamire, 2013).
- The time of day that the images are acquired. Gray matter volume decreases significantly from morning to afternoon, mostly in frontal and temporal regions (Trefler et al., 2016). This phenomenon is found regardless of whether the VBM or Freesurfer technique are used to analyse images.
- The choice of method for pre-processing and analysis. The three most widely used softwares for volumetric analysis are Statistical Parametric Mapping (SPM), FMRIB Software Library (FSL), and Freesurfer. The automated segmentation differs and can cause different volumetric measures. The largest differences are found in cortical volumes whereas sub-cortical structures are more similarly assessed across these methods (Popescu et al., 2016).

### **1.2.3 The dorsolateral prefrontal cortex**

The dorsolateral prefrontal cortex (DLPFC) is located in the frontal lobes, rostral to the premotor cortex. This brain region is connected to the auditory, visual, and somatosensory systems. It is also strongly connected to the anterior cingulate cortex (ACC). The primary function of the DLPFC is to process high level sensory information, for example motion and spatial dimensions of vision, and, through connections with the hippocampus, integrate this information into a context. Through the connections with the medial prefrontal cortex, this context is labelled with an emotion. Many illnesses that present with behavioral and cognitive

deficits have been associated with volumetric alterations in the DLPFC: frontotemporal dementia (Pan et al., 2012), Alzheimer's dementia (Byun et al., 2015), schizophrenia (Torres et al., 2016), and also bipolar disorder (Lyoo et al., 2006).

#### **1.2.4 The medial prefrontal cortex**

The medial prefrontal cortex (mPFC) is involved in top-down regulation of emotional processes (Savitz, Price, & Drevets, 2014b). One of its main functions is to integrate input of context and events and, based on memory, output signals that will lead to the most favorable outcome (Euston, Gruber, & McNaughton, 2012). Context, in this case, also involves emotion based on somatosensory stimuli that has been processed in the insula. Emotional top-down control is mostly executed from the ventral mPFC while the dorsal mPFC exerts motor control. One of the key areas of the mPFC is the anterior cingulate cortex. It is active in several processes that require inhibition of responses. The rostral part of the mPFC, the frontal pole, is involved in abstract thinking, meta cognition, and self-referential mentalizing (van Veluw & Chance, 2014). Neuroimaging and post-mortem histopathological studies have shown differences in mPFC between persons with bipolar disorder and healthy controls, see table 1.1.

Table 1.1. Summary of the *postmortem* and the neuroimaging findings in BD.

	Region				
	sgACC	pgACC	Supragenual ACC	OFC	BA 9
Glutamatergic neuron density	↓ +	–	–	–	↓ +
Glutamatergic neuron number	–	–	–	–	–
Glutamatergic neuron size	↓ +	–	–	–	↓ +
GABAergic neuron density	↓ +	↓ ++	↓ +	↑ +	↓ ++
GABAergic neuron number	–	↓ +	–	–	↓ +
GABAergic neuron size	–	–	–	–	–
Glial cell density	–	–	–	–	↓ ++
Glial cell number	↓ +	–	–	–	↓ +
GM volume	↓ ++	↓ +	–	↓ ++	–
WM pathology (DTI)	++	–	–	+	–
Metabolic (MRS)	–	+	–	–	+

*Notes: sgACC = subgenual anterior cingulate cortex, pgACC = pregenual ACC, OFC = orbital frontal cortex, BA 9 = Brodmann's area 9, DTI = diffusion tensor imaging, MRS = magnetic resonance spectroscopy.*

*↑ increased, ↓ decreased, + modest evidence, ++ strong evidence, – no or minimal evidence.*

From: Savitz J, Price J, Drevets W. *Neuropathological and neuromorphometric abnormalities in bipolar disorder: View from the medial prefrontal cortical network.* Neuroscience and biobehavioral reviews. 2014; 42; 132-147.

### 1.2.5 The fronto-insular network

The insula is the fifth and smallest lobe of the brain. It is located beneath the frontal, temporal, and parietal lobes. It is divided into a posterior and an anterior part which are divided by a sulcus that is aligned with the central sulcus of the brain surface. The anterior and posterior parts are cytoarchitectonically different and connect to different cortical and subcortical regions. The anterior insula is connected to the prefrontal, orbitofrontal, temporopolar, anterior cingulate areas, as well as to the somatosensory and opercular areas of the parietal lobe. It is also connected to the amygdala, anterior hippocampus, and the brainstem. The posterior insula shares connections with limbic structures and primary somatosensory regions.

The anterior insula is activated when feelings are induced, such as anger, sexual arousal, trust and disgust (Craig, 2002). It is a key cortical area in the interoceptive network where it monitors the physiological status of the body. Through its connection with medial prefrontal areas, such as the ACC, it generates a mental image of the state of the body. Both the anterior insula and ACC are activated in situations that involve social errors and induce feelings such as guilt (Shin et al., 2000), resentment (Sanfey, Rilling, Aronson, Nystrom, & Cohen, 2003), embarrassment (Berthoz, Armony, Blair, & Dolan, 2002), and deception (Spence et al., 2001). The role of this network is believed to detect social errors and adapt the responses to them.

In major depression, the activity in the right anterior insula has been shown to predict treatment response. Interestingly, high glucose metabolism in this area has been associated with response to escitalopram, whereas hypometabolism have been associated with response to cognitive behavioral therapy (McGrath et al., 2013).

## 1.3 MAGNETIC RESONANCE IMAGING

In a MRI scanner, a large magnet aligns the water molecules (e.g., hydrogen  $^1\text{H}$ -protons) in the tissue and makes them oscillate between being aligned with the magnetic field or in the opposite direction. A larger magnet causes more protons to be aligned *with* the magnetic field. The protons are spinning around an axis that is close to parallel to the magnetic field. The slight difference in the alignment of the axis and the magnetic field makes the protons precess (i.e. wobble) with a certain frequency. When a radio signal (RF) with this frequency is turned on, the  $^1\text{H}$ -protons absorb energy and flip their spin. When the signal is turned off, the protons are again realigned with the magnetic field. During this process called relaxation, the protons release a radio frequency signal that can be picked up by a coil. The varying degree of water content in different tissues produces different signal strength, intensity.

In our studies, we used T1-weighted MRI-images. T1 is the time it takes for a substance to become longitudinally realigned to the magnetic field following the RF pulse. A short time



gives a low signal, making small, free flowing molecules like water appear dark in the images.

## **1.4 METHODS FOR ANALYZING BRAIN VOLUMES**

The MRI-images are made up by box-shaped units called voxels. Each voxel has a surface area, defined by the resolution of the image, and a height, defined by the slice thickness. The average intensity in the volume of the voxel gives the voxel value.

### **1.4.1 Region of Interest-analyses**

The early studies of CT and MRI images were regions of interest (ROI)-analyses. A ROI analysis required that the investigator manually measured a chosen region in the brain for all subjects, calculated the volume, and then performed the statistical analyses. This is labor intensive and time-consuming work but the anatomical regions were defined with high accuracy. Contemporary software for image analysis, such as Freesurfer, can provide automated labelling of a number of pre-defined regions with an accuracy that is equal to manual measurements (Fischl et al., 2002). An advantage of ROI analyses is that they are not as easily confounded by magnetic field heterogeneity as whole-brain analyses. The downside of ROI-analyses is that if the ROIs are too large, volumetric differences within a part of the ROI may be missed. It is also impossible to tell where within the ROI the difference is located. If the ROIs are too small, differences outside the ROIs will be missed. Alternatively, one must include several ROIs which cause loss of statistical power due to correction for multiple comparisons.

### **1.4.2 Voxel-Based Morphometry**

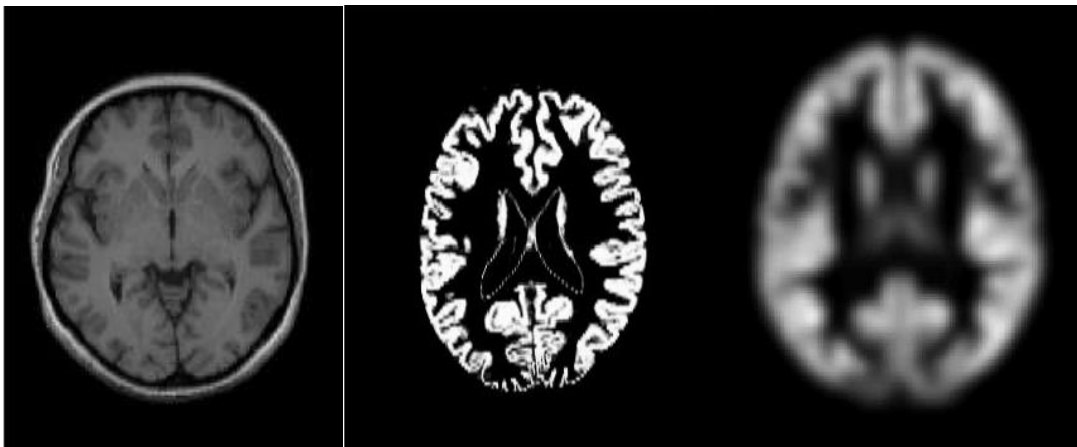
Voxel-based morphometry (VBM) is an automated method for analyzing volumes in the brain. It was developed by Karl Friston and John Ashburner at UCL in London, UK. The computer software that is used is called Statistical parametric mapping (SPM) (Ashburner & Friston, 2000, 2005).

The VBM method is based on the idea that each voxel belongs to one tissue class and that the tissue in the voxel has a volume different from the surface x slice thickness volume. To enable this, pre-processing of the images is necessary. Group analyses namely require that all the brains have the same size and shape and that the tissue class of each voxel is defined (gray matter, white matter, and cerebrospinal fluid (CSF)). When the pre-processing is done, each voxel has a value between 1 and 0 that represents the volume of that voxel.

The basic preprocessing steps of VBM are to:

- spatially normalize and warp all brain images to the same space. This is done by registering each image to a template by minimizing the residual sum of squared difference between template and image. Normalization means that some brain areas are expanded and others contracted. To compensate for this, and keep the original volume in the expanded or contracted areas, the images are modulated. Modulation means that the voxel value is scaled by the amount of expansion or contraction.
- segment the images into three tissue classes (gray, white, CSF) by registering the spatially normalized images to tissue probability maps.
- smooth the images. By applying a three-dimensional Gaussian kernel to the segmented images, the variance becomes more normally distributed and inhomogeneity from the spatial normalization is compensated for.

Contemporary versions of SPM have integrated the normalization and segmentation steps. These versions also enable the use of DARTEL (A fast diffeomorphic image registration algorithm) to register images with higher precision (Ashburner, 2007).

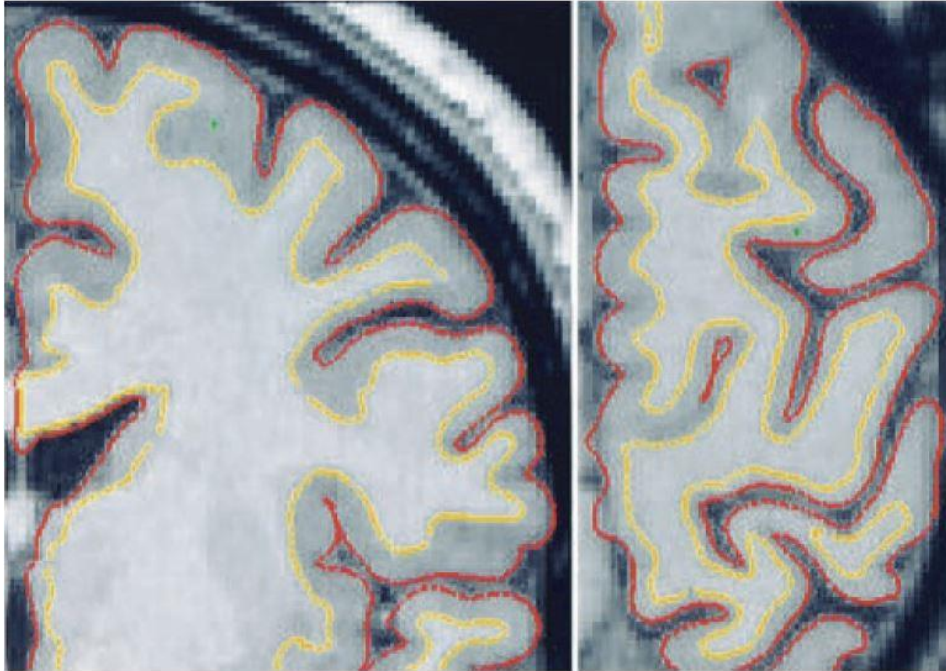


*Figure 1.1. Preprocessing of images for VBM-analyses. From left to right: Raw T1 image, segmented gray matter image, normalized and smoothed gray matter image.*

### 1.4.3 Freesurfer

Freesurfer is also an automated method for analyzing MRI-images on group level. Freesurfer has two different ways of pre-processing the images. The volume-based method for subcortical analyses will not be discussed here since it is not used in our studies. For analyses of cortical volumes, the cortical surface reconstruction method is most commonly used. Here, the images are first registered to a standard template. Then the white matter surface and the border between gray matter and CSF are outlined by calculations of differences in intensity between the different tissues (Figure 1.2). Hence a gray matter image is created. The image

contains a large number of nodes based on the position of different structures in the template. By interconnecting these nodes a network is created that enables the cortical surface to be inflated to a smooth surface with no gyri or sulci. This surface can then be anatomically parcellated by registering it to a probabilistic atlas (Desikan et al., 2006), thus creating 68 anatomical regions. Then the cortical surface area of each region can be measured. To obtain values for cortical thickness, the distance between the gray/white matter boundary and the gray/CSF boundary is measured and the average thickness value for each region is calculated.



*Figure 1.2. Coronal (Left) and horizontal (Right) slices of the left hemisphere with Gray/white (yellow) and pial (red) surfaces overlaid (Fischl & Dale, 2000).*



## 2 AIMS

The overarching aim of this thesis was to advance the knowledge about the volumetric changes in brain gray matter in bipolar disorder and their association with specific symptoms and illness progression. Our specific aims were:

- to analyze potential differences in gray matter volume, on group level, between persons with bipolar disorder and healthy controls.
- to analyze the association between gray matter volume and illness progression, i.e., the duration of illness, the number of lifetime manic and depressive episodes, in persons with bipolar disorder.
- to further investigate volume changes related to the number of manic episodes in a 7-year follow up to be able to separate causes from effects.
- to investigate gray matter volume changes associated with psychosis in people with bipolar disorder.



## **3 MATERIAL AND METHODS**

### **3.1 THE ST. GÖRAN BIPOLAR PROJECT**

The St. Göran Bipolar Project (SBP) is a longitudinal, observational study of bipolar patients that started in 2005 at Affektivt Centrum, which is a tertiary out-patient unit for people with bipolar disorder at the Northern Stockholm Psychiatric Clinic in Sweden. All patients diagnosed with bipolar disorder at this unit were eligible for study inclusion. All studies in this thesis are based on data from the SBP.

#### **3.1.1 Assessment of diagnosis**

The patients were examined by a psychiatrist or resident in psychiatry. A semi-structured protocol was used to establish the diagnosis. This protocol is a Swedish version of the Affective disorder evaluation (ADE) protocol that was used in the STEP-BD project (Sachs et al., 2003). The ADE contains a social anamnesis, medical history, and the affective module of the Structured Clinical Interview for DSM-IV (SCID). It also includes a detailed rating of symptoms during manic and depressive episodes, as well as an assessment of previous psychotic symptoms. Comorbidity was assessed by using the M.I.N.I. International Neuropsychiatry Interview. Several self-rating scales, including the AUDIT and DUDIT were used to screen for substance misuse. All information collected from the examination, medical charts, and interviews with next of kin when available were presented at a diagnostic case conference with 2-5 psychiatrists present. A best-estimate diagnostic decision was made and patients fulfilling criteria for bipolar disorder type I, II, not otherwise specified (NOS), and schizoaffective disorder bipolar type were invited to participate in the study.

The number of participants differs across the studies in this thesis. Since study IV had been completed, additional patients have been included. Study I, II, and III differ due to different exclusion criteria for each analysis, respectively. Study V has a lower number of participants because all follow-up examinations had not been completed at the time of the study.

#### **3.1.2 Healthy controls**

Healthy controls were recruited by letting Statistics Sweden send letters with an invitation to participate to sex- and age matched, randomly selected, individuals from the same catchment area. Seven potential control subjects were selected for each patient and the response rate was 14%. Responders were first screened for mental illness and other medical conditions by a research nurse through a telephone interview. Eligible persons were then scheduled for an

interview and examined by a psychiatrist using the M.I.N.I. and selected parts of the ADE to exclude psychiatric disorders.

## **3.2 MAGNETIC RESONANCE IMAGING**

### **3.2.1 Image acquisition**

MRI scans were acquired in a General Electric Signa Excite 1.5T scanner at the Karolinska MR Research Centre from January 2006 to December 2011. Scans for the 7-year follow up were acquired in the same scanner. Sagittal T1 weighted, axial and coronal T2 weighted, and axial fluid attenuation inversion recovery (FLAIR) T2 weighted scans were acquired for examination by a senior radiologist to screen for clinically significant anatomical abnormalities. For the volumetric analyses, all subjects were examined in the same scanner and with the same protocol, using an eight-channel head coil. 1.8mm slices of coronal images were acquired using a three-dimensional spoiled gradient echo recall sequence (3D-SPGR) with the following parameters: time to repetition=21.0ms, echo time=6ms, number of excitations=1, flip angle=30°, acquisition matrix=256x256x124. Two 3D-SPGR series of images were acquired for each subject. Both patients and control were scanned in the afternoon at approximately the same time of day (4 pm).

### **3.2.2 Voxel-Based Morphometry**

In studies I, III and IV in this thesis, we used the VBM method. In study I, we used the software version SPM8, in study III the SPM12 version, and in study IV the SPM5 version. SPM5 differs in the pre-processing from the other two versions. In study IV, we also used the VBM-Toolbox (Gaser, 2009). In study I and III, all images were manually aligned to the anterior-posterior commissure line by the same investigator (C-J.E.). Images were then segmented into gray matter, white matter, and CSF in native space. The segmented images were rigidly aligned to the tissue probability map, which enables the use of DARTEL. A mean template was then created from the rigidly aligned gray and white matter images from all subjects. Then the deformations from the template to each individual image were computed and the deformations were saved as “Flow fields”. Using the flow fields, the gray matter images were warped to match the template and normalized to MNI-space. Finally, the warped and normalized images were smoothed by applying a three-dimensional Gaussian kernel on all voxels. In study I and III, we used a 8x8x8mm smoothing kernel.

In study IV, the pre-processing differed from the other studies. Here, we used two series of 3D-SPGR images for each subject. The two series of images were realigned and resliced to yield a mean image for each subject. All images were then manually aligned to the anterior-posterior commissure line by the same investigator (C-J.E.). The mean images were then segmented into



gray and white matter images based on a tissue probability map. The gray matter images were then modulated and normalized to MNI-space. Finally the images were smoothed. In study IV, we used a 10x10x10mm full width at half maximum Gaussian smoothing kernel.

Voxel-wise mass univariate analyses were performed by linear multiple regression with gray matter volume as the dependent variable. Continuous variables or group were used as covariates, and different covariates of no interest were added. Please see Study I-V for details.

### **3.2.3 Freesurfer**

In study II and V, the MRI-images were analyzed using Freesurfer v5.1. The images were segmented into cortical gray matter images and the values for cortical thickness and surface area were obtained using the semi-automated cortical surface reconstruction method (Dale, Fischl, & Sereno, 1999; Fischl & Dale, 2000; Fischl, Sereno, & Dale, 1999). This method strips the skull from the images, defines the border between gray and white matter as well as the border between gray matter and CSF. The cortical gray matter images are then smoothed, transformed, and resampled into a standard space.

## **3.3 ETHICS**

All studies were conducted in accordance with the Helsinki declaration and approved by the Regional Ethic Review Board in Stockholm, Sweden. All patients and controls consented orally and in writing to participate in the study after have been given a full disclosure of the study procedures.



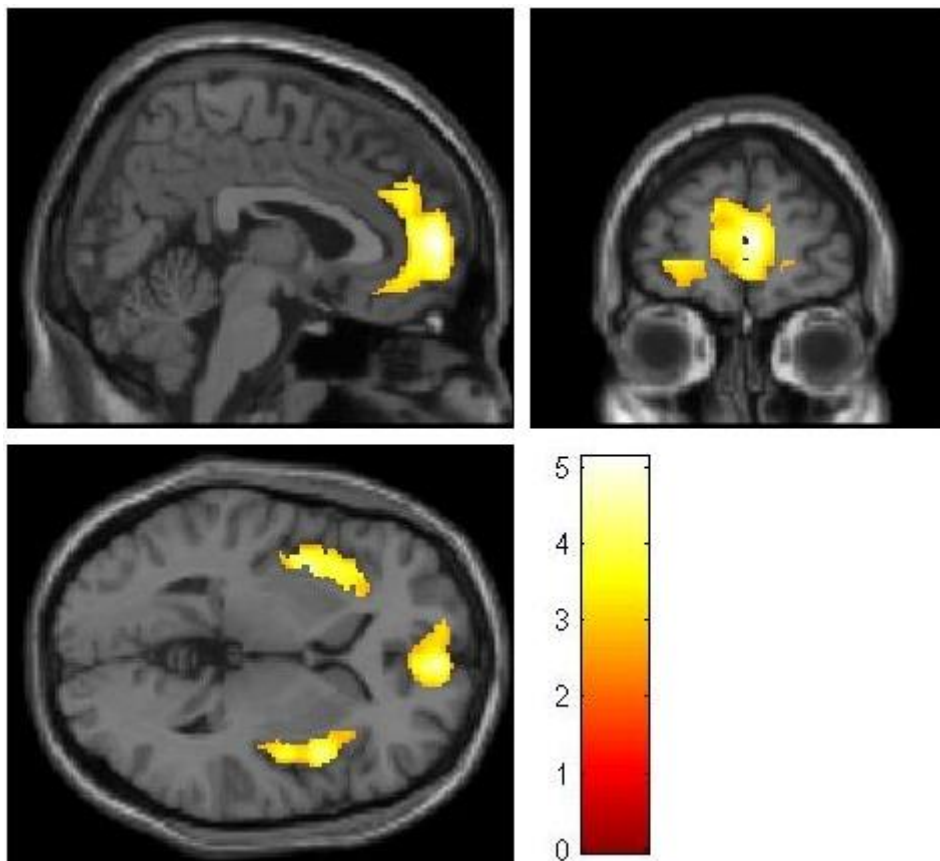
## 4 RESULTS AND DISCUSSION

### 4.1 STUDY 1

#### 4.1.1 Gray matter volume in bipolar patients and healthy controls

We analyzed brain gray matter volume difference between 219 patients with bipolar disorders (types I, II and Not Otherwise Specified) and 102 healthy controls using a voxelwise mass-univariate analysis (VBM) of MRI images. We corrected the analysis for age, sex, and scanner filter. In a post-hoc analysis, we also corrected for bipolar subtype, comorbid ADHD, medication, body mass index, and level of education. The main results were still statistically significant after controlling for these confounders ( $p < 0.05$ , FWE-corrected on voxel level).

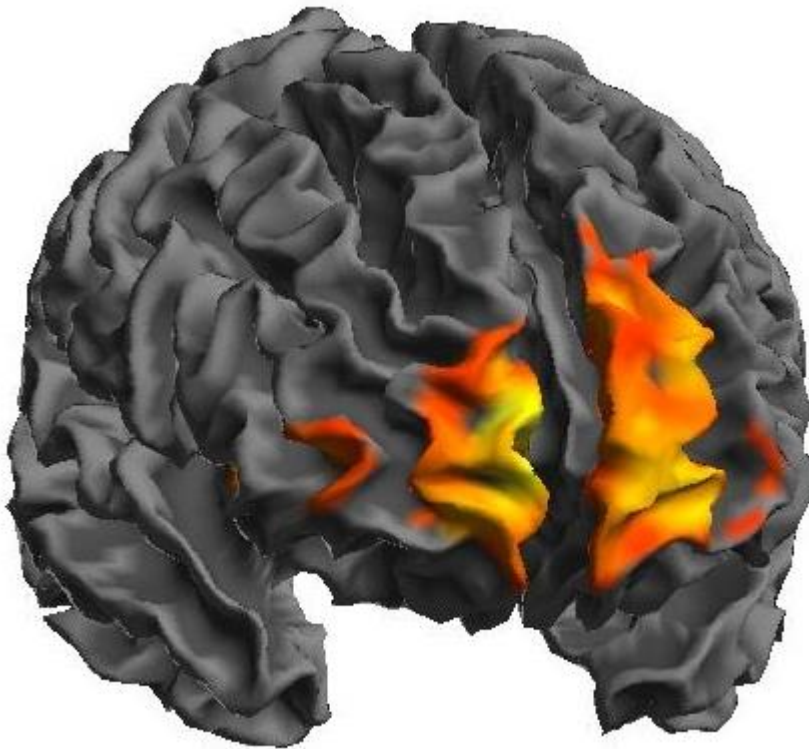
We found that patients with bipolar disorder had smaller gray matter volume in the right insula (peak voxel  $X=44$   $Y=12$   $Z=-3$  MNI-space, FWE-corrected peak voxel  $p=0.001$ ,  $Z$ -value= 4.98, cluster size 111) , left insula (peak voxel  $X=-39$   $Y=17$   $Z=-3$ ,  $p=0.002$ ,  $Z=4.81$ , 98 voxels), and the right medial prefrontal cortex (peak voxel  $X= Y=59$   $Z=6$ ,  $p=0.001$ ,  $Z=5.01$ , 416 voxels) compared with healthy controls (Figure 4.1).



*Figure 4.1. Insular and frontal gray matter volume reduction in patients with bipolar disorder. Colored bar represents T-score. For illustrational purposes, statistical significance threshold was set to  $p < 0.005$  uncorrected.*

#### 4.1.2 Analysis of volumetric correlation

The voxel of peak difference in the right insula was located in the anterior part, MNI-space coordinates  $X=44$   $Y=12$   $Z=-3$ . We extracted the volume in that voxel for each subject and entered it as a covariate in a whole-brain regression analysis with gray matter volume as the dependent variable. This was done in order to investigate if there was any other area in the brain which volume was linearly co-varying with the volume in the right anterior insula. We thus found a volumetric correlation with the left insula (not shown) and with a large part of the rostral and medial prefrontal cortex (Figure 4.2).

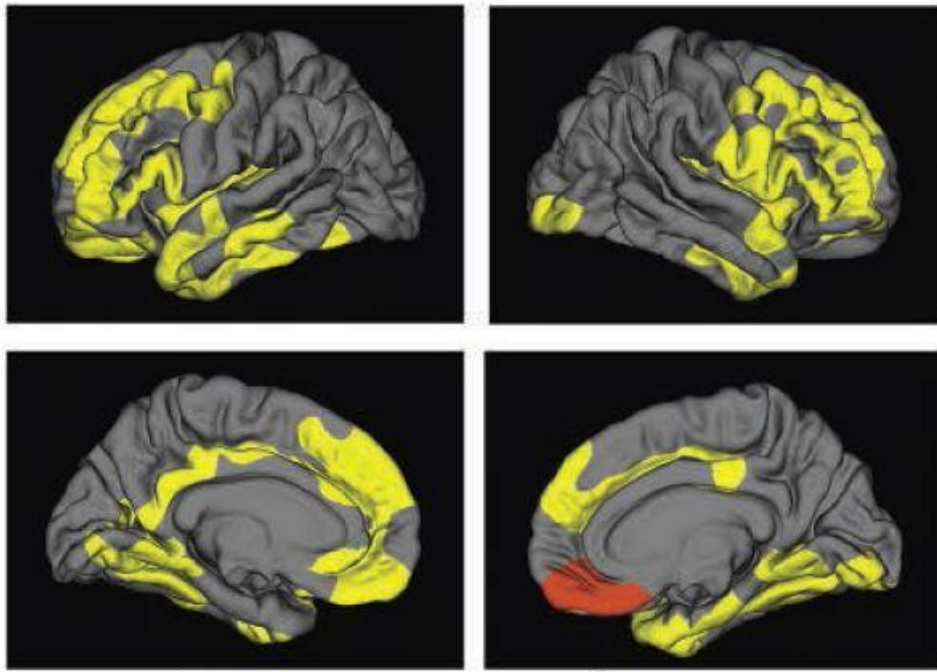


*Figure 4.2. Regions of the prefrontal cortex that co-varies in volume with the right anterior insula.*

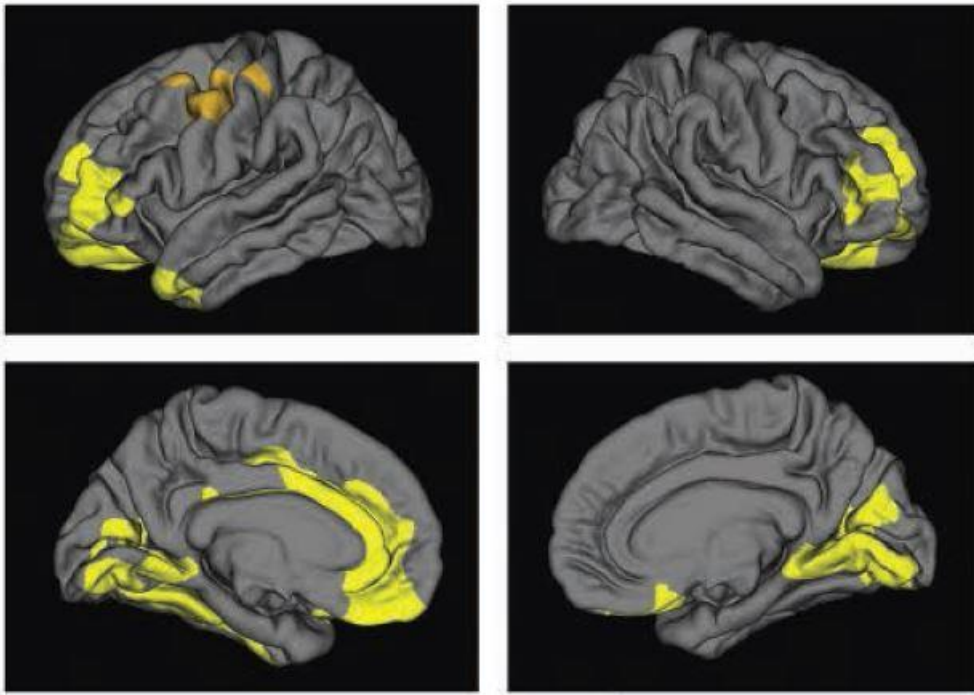
The result of reduced volume in the right anterior insula corroborates a meta-analysis study (Selvaraj et al., 2012). This meta-analysis also showed suggestive – but inconclusive – reduction of gray matter volume in the medial prefrontal cortex. Hence, our results add further evidence to that insular volume is reduced in bipolar disorder. We also verify that the medial prefrontal cortex is reduced in volume. These regions are strongly coupled to emotional processing. We can also demonstrate that the insula and the mPFC are volumetrically correlated. This suggests that it may be the same neuropathological causes to the volume reduction in these two regions.

## 4.2 STUDY 2

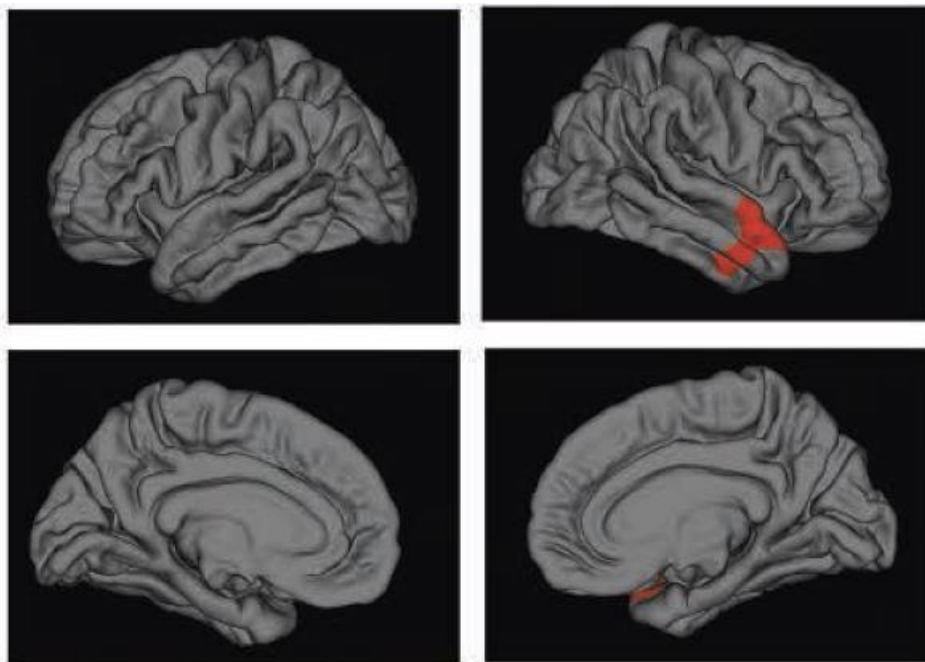
In study 2, we compared the bipolar subtypes I and II. Using Freesurfer, we performed whole-brain analyses of cortical thickness, surface area, and volume in 81 patients with Bipolar I disorder, 59 with Bipolar II disorder, and 85 healthy controls. We found that both the bipolar I and II groups had smaller cortical volume in several prefrontal areas compared with healthy controls. The bipolar I group also showed smaller volume in temporal, occipital and parietal regions compared with controls. Further, the bipolar I disorder group had thinner rostral temporal cortex compared with the bipolar II disorder group.



*Figure 4.3. Smaller volume in the bipolar I disorder sample compared with controls, corrected for multiple comparisons (Abe et al., 2016).*



*Figure 4.4. Smaller volume in the bipolar II disorder sample compared with healthy controls, corrected for multiple comparisons (Abe et al., 2016).*



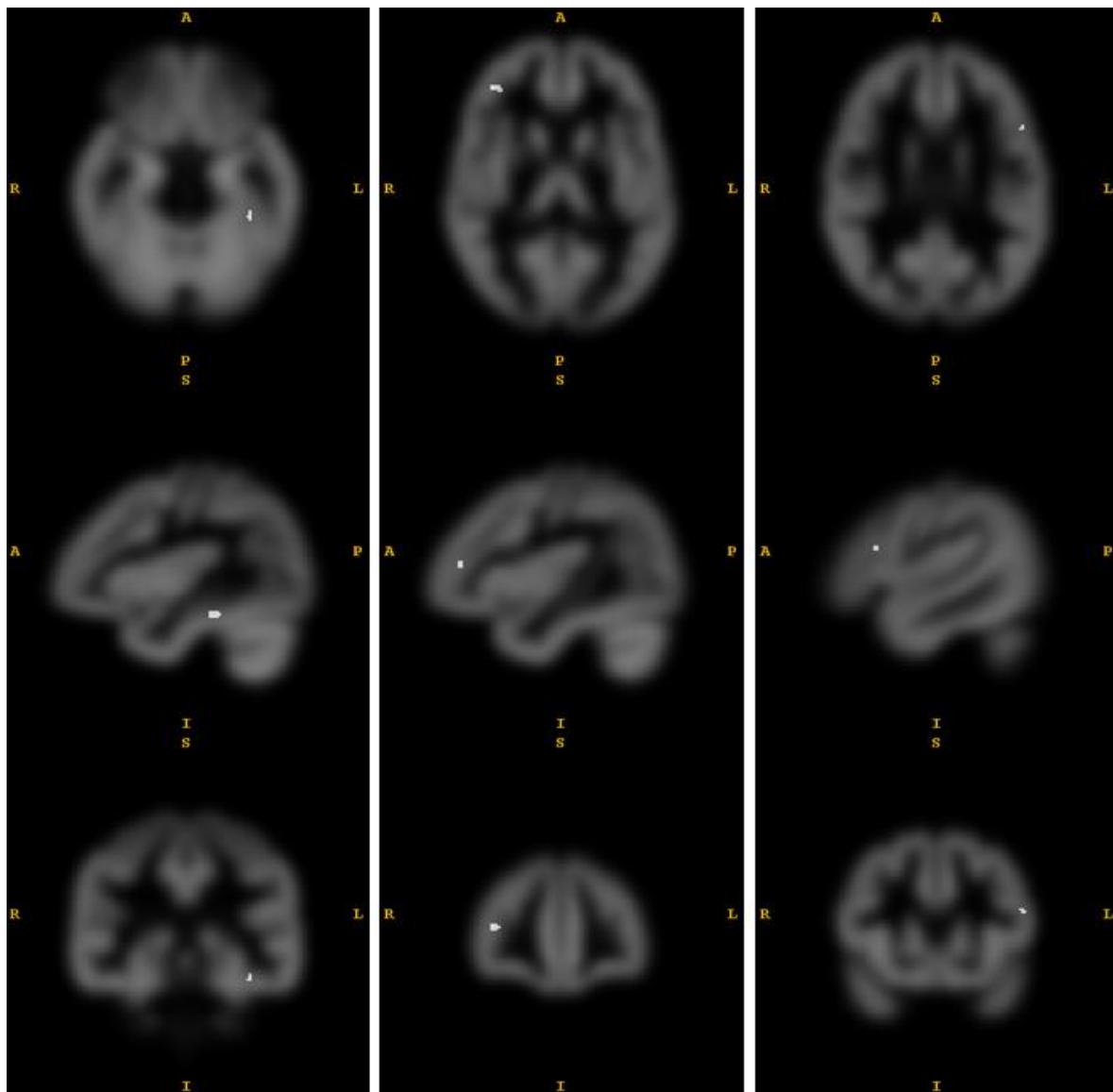
*Figure 4.5. Thinner cortex in the bipolar I disorder sample compared to the bipolar II disorder sample, corrected for multiple comparisons (Abe et al., 2016).*

This study shows reduced cortical volume in the same regions as Study 1, but also in additional prefrontal, parietal, and occipital regions. Hence, two studies using different methods of image analysis show reduced volume in the medial prefrontal cortex. This study also corroborates the results of another large study of similar design (Rimol et al., 2012).

Compared with controls, the bipolar I group showed areas of low volume that was shared with, but larger than, the bipolar II group (Figures 3 and 4). This indicates similar underlying neuropathology in the two subgroups of bipolar disorder. The difference between the two groups may reflect the more severe symptoms in mania and depression in bipolar I disorder.

### **4.3 STUDY 3**

In study 3, we aimed to analyze gray matter volume changes associated with a history of psychotic symptoms. In order to exclude disease-specific findings, we performed a case-case analysis of patients with bipolar disorder who had and had not experienced psychotic symptoms during mood episodes. Using VBM, we thus compared gray matter volume in 85 individuals with a history of psychosis with 82 individuals without a history of psychosis. Level of statistical significance was set to  $p < 0.05$ , family-wise error corrected on voxel level. In the psychosis group, we found smaller gray matter volume in the right DLPFC (peak voxel X=39 Y=42 Z=11 MNI-space, FWE-corrected  $p=0.014$ ,  $T=4.98$ , cluster size 33 voxels), the left fusiform gyrus (peak voxel X=-38 Y=-33 Z=-20  $p=0.010$ , cluster size 18 voxels,  $T=5.07$ ) and the left inferior frontal gyrus (peak voxel X=-51 Y=18 Z=21,  $p=0.033$ ,  $T=4.59$ , 9 voxels). The volumes in these areas were also smaller when compared with healthy controls whereas the no-psychosis group did not differ from healthy controls. The patient groups differed with respect to the distribution of bipolar disorder subtype. Bipolar I disorder was more common in the group with a history of psychosis, while a majority of patients in the no psychosis group had bipolar II disorder.



*Figure 4.6. Regions of smaller gray matter volume in patients with bipolar disorder and a history of psychosis compared with bipolar patients with no history of psychosis.  $p < 0.05$  FWE-corrected (C. J. Ekman et al., 2016).*

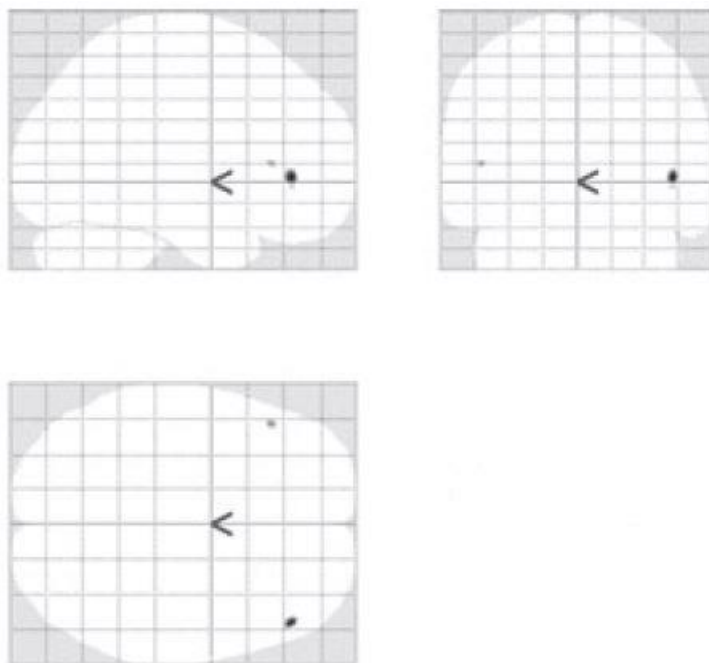
The regions of reduced gray matter volume are not the same as the ones Study 1 demonstrated to be associated with bipolar disorder. This strengthens the probability that these regions are associated specifically with psychotic symptoms rather than with bipolar disorder in general. Moreover, the three regions of reduced volume have been associated with psychotic symptoms in other studies. The DLPFC has been associated with psychosis in functional MRI-studies where it shows altered signaling in prediction error tasks (Corlett et al., 2006; Corlett et al., 2007). The volume of the fusiform gyrus has been shown reduced in schizophrenia and the reduction has also been associated with more positive (hallucinatory/delusional) symptoms (Song et al., 2015). Reduced volume in this region has also been shown in persons with auditory hallucinations who do not fulfill the criteria for schizophrenia (van Lutterveld et al., 2014). The inferior frontal gyrus has lower volume in schizophrenia patients with hallucinations than those without (van Tol et al., 2014). Hence, reduced gray matter volume in all three regions evidently can contribute to the development



of psychotic symptoms. We wanted to perform an analysis within the psychosis group, comparing those patients who had experienced hallucinations with those who had had delusions. Unfortunately too few individuals had had hallucinations without delusions so we did not have the statistical power to perform this analysis. The potential correlation between brain structure and different symptoms of psychosis is, though, a very interesting topic that needs to be investigated further.

#### 4.4 STUDY 4

In study 4, we examined the relationship between gray matter volume and illness progression. The study included 55 patients from the St Göran Project. All patients had Bipolar I disorder and did not fulfill criteria for any other psychiatric illness in the M.I.N.I. screening. Using VBM, we performed mass-univariate linear regression analyses with gray matter volume as the dependent variable and either lifetime manic episodes, lifetime depressive episodes, or illness duration as covariates. Statistical significance was defined as  $p < 0.05$  family-wise error corrected on voxel level. We found a negative correlation between the lifetime number of manic episodes and gray matter volume in the right DLPFC (peak voxel  $X=47$   $Y=40$   $Z=3$ ,  $p=0.0004$ ,  $T\text{-score}=6.79$ , cluster size 144 voxels) and the left DLPFC (peak voxel  $X=-47$   $Y=30$   $Z=9$ ,  $p=0.011$ ,  $T=5.75$ , cluster size 38). We found no significant correlation between gray matter volume and the number of depression episodes or illness duration.



*Figure 4.7. Clusters of gray matter volume reduction that were linearly correlated to the lifetime number of manic episodes (C. J. Ekman, Lind, Ryden, Ingvar, & Landen, 2010).*

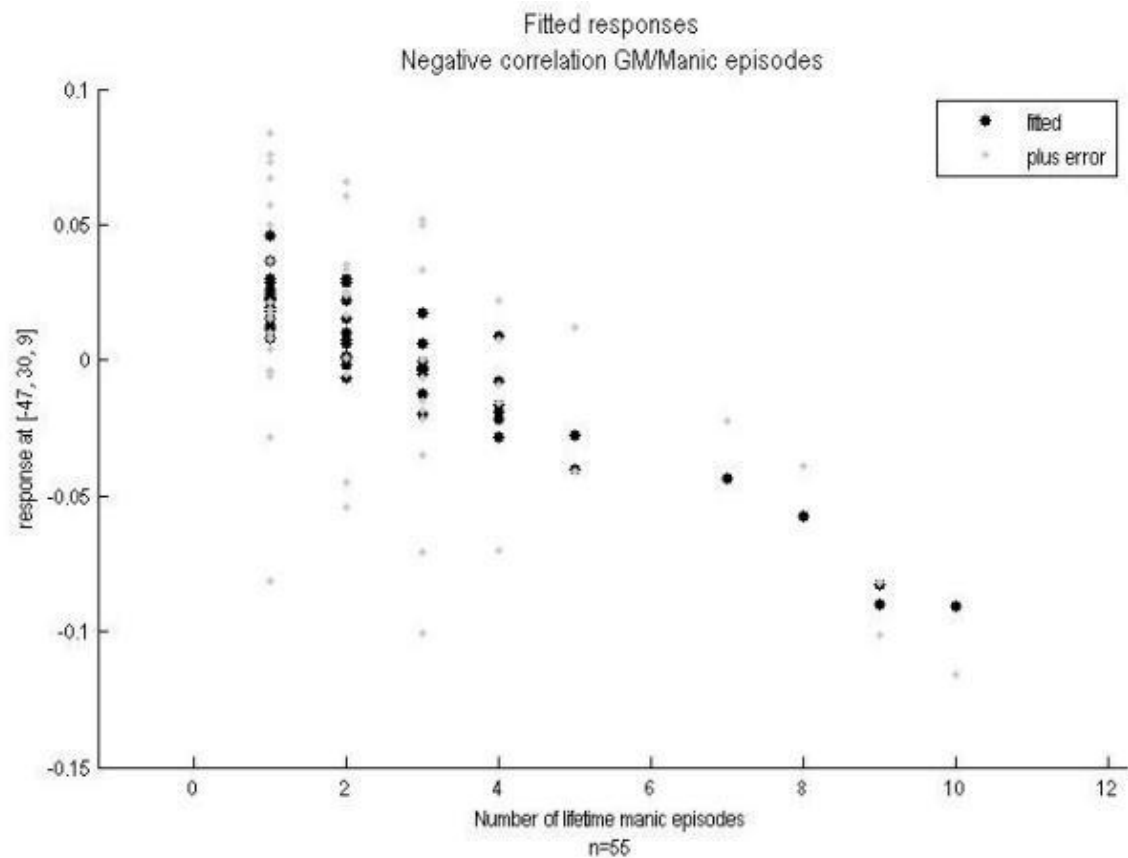


Figure 4.8. Correlation between the lifetime number of manic episodes and the gray matter volume in the right dorsolateral prefrontal cortex.

In this study, we found a linear correlation between the lifetime number of manic episodes and the gray matter volume of the DLPFC bilaterally. The cross-sectional design of the study does not make it possible to tell if the volume reductions cause a more severe illness with frequent manic episodes or if the reductions are caused by the manic episodes.. Interestingly, we found no correlation between gray matter volume and the lifetime number of depressive episodes. Imaging studies of unipolar depression have shown reduced GM volume in several cortical and subcortical regions (Arnone, McIntosh, Ebmeier, Munafo, & Anderson, 2012). One of the reasons we failed to demonstrate any association between GM volume and the number of depressive episodes may be methodological: The reported lifetime number of episodes has proven difficult to estimate by interviewing patients and we found much greater variability in the self-reports of depression than on mania.

## 4.5 STUDY 5

The results from Study 4 do not tell if the reduced DLPFC volume is causing higher numbers of manic episodes, or if it is an effect of multiple manic episodes. To further evaluate the cause or effect question, we analyzed MRI images from the 7-year follow up scans in the SBP. Out of 31 patients with bipolar disorder type 1 who at the time had been scanned at both

timepoints, 13 had had a manic episode during the six years whereas 18 did not. Using Freesurfer, we thus compared cortical thickness, volume and surface area in the DLPFC and inferior frontal cortex (INFRO) between the two groups at both timepoints. The regions of interest were automatically parcellated in Freesurfer. There was no difference in thickness or surface area at baseline. At follow up, the group with manic episodes demonstrated a significantly smaller volume in both the DLPFC and the inferior frontal cortex (Figure 4.9 and table 4.1).

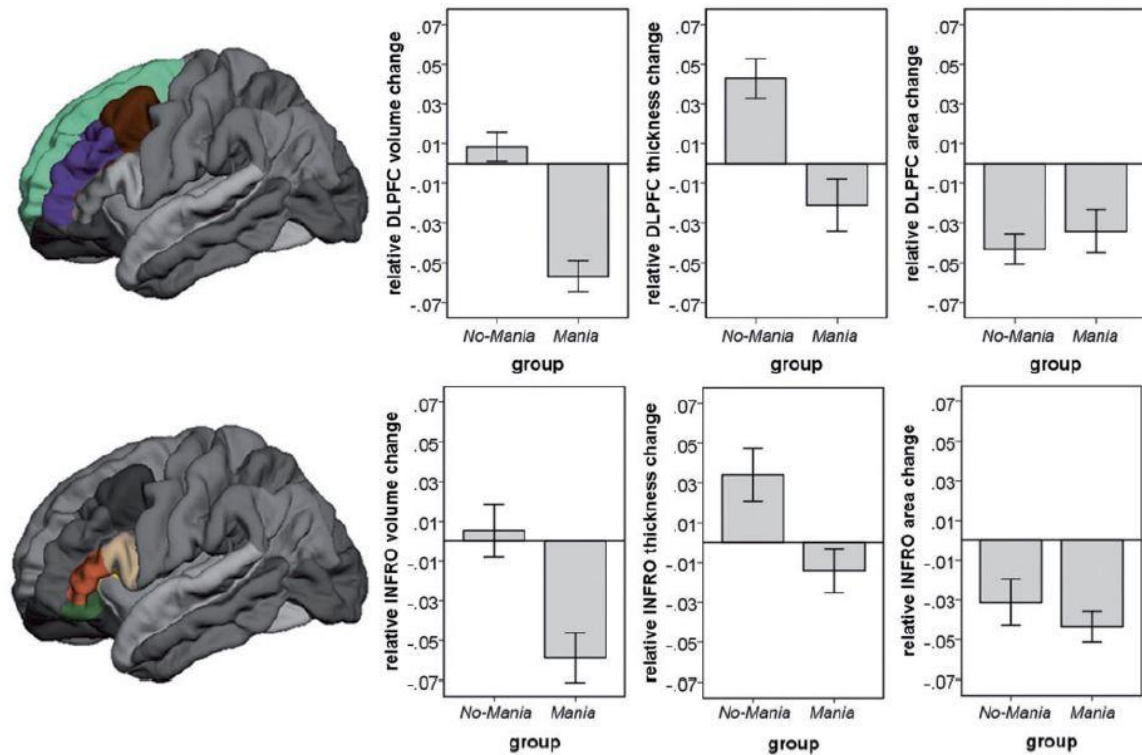


Figure 4.9. Relative change in dorsolateral prefrontal cortex (DLPFC) and inferior frontal cortex (INFRO) volume, thickness, and surface area between the mania and no-mania groups at follow-up (Abe et al., 2015).

Table 4.1. Main results for group differences in DLPFC and inferior frontal cortex

		No-Mania Group		Mania Group		No-Mania versus Mania	
Region	Measure	Mean $\pm$ SD	P (t)	Mean $\pm$ SD	P (t)	P	ES
DLPFC	Volume	0.005 $\pm$ 0.045	NS (0.8)	-0.052 $\pm$ 0.042	<0.001 <sup>*</sup> (-4.9)	<0.001 <sup>*</sup>	1.64
	Thickness	0.039 $\pm$ 0.042	<0.001 <sup>*</sup> (4.3)	-0.015 $\pm$ 0.048	NS (-1.6)	<0.001 <sup>*</sup>	1.62
INFRO	Area	-0.041 $\pm$ 0.033	<0.001 <sup>*</sup> (-5.6)	-0.038 $\pm$ 0.039	0.008 <sup>*</sup> (-3.2)	NS	0.09
	Volume	0.003 $\pm$ 0.051	NS (0.4)	-0.055 $\pm$ 0.051	<0.001 <sup>*</sup> (-4.8)	0.004 <sup>*</sup>	1.14
INFRO	Thickness	0.031 $\pm$ 0.047	0.021 (2.55)	-0.011 $\pm$ 0.047	NS (-1.3)	0.024	0.89
	Area	-0.030 $\pm$ 0.042	0.015 <sup>*</sup> (-2.70)	-0.045 $\pm$ 0.043	<0.001 <sup>*</sup> (-5.7)	NS	0.35

*Results of main analysis on DLPFC and inferior frontal cortex (INFRO) regions of interest . Within-group means ( $\pm$ SD) of the per cent change in cortical thickness, area, and volume are given, along with P-values and t-statistics of one-sample t-tests (df = 12 in Mania, df = 17 in No-Mania). Right column: group comparison of the means (ANCOVA analysis), corresponding P-value, and Cohen's d effect size. ES = effect size; NS = not significant.*

*\*Results significant after correction for multiple comparisons. Adjusted alpha levels were 0.020 (No-Mania) and 0.019 (Mania) for the within-group t-tests; and 0.021 for the group comparison (No-Mania versus Mania).*

This study strongly suggests a causal relationship between manic episodes and gray matter reduction in the dorsolateral prefrontal cortex and the inferior frontal cortex. It also shows

that the volume reduction is caused by both cortical thinning and a decrease in surface area. Interestingly, cortical thickness is increased compared to baseline in subjects that did not have any manic episodes during the follow-up period. Given the fact that all of these individuals have had at least one manic episode prior to the baseline scanning, this cortical thickening can be interpreted as if the brain slowly heals during the euthymic phase.

An effect of reduced volume could be seen even when excluding patients with multiple manic episodes, which means that one single manic episode cause a significant decrease in cortical volume. Cognitive deficits in bipolar disorder have been correlated with the number of hospitalizations in other studies. The DLPFC is a region involved in executive function. Hence, the gray matter volume reduction in DLPFC, caused by manic episodes, may be a neuropathological correlate to cognitive deficits.



## 5 GENERAL DISCUSSION

The studies in this thesis describe volumetric alterations in the brain in persons with bipolar disorder. In **Study 1**, we found differences between bipolar disorder patients and healthy controls. In **Study 2**, we found differences between patients with bipolar disorder I and II, which were not associated with bipolar disorder per se. **Study 3** described volumetric changes associated with psychotic symptoms in bipolar disorder subjects. In **Study 4**, we found a volume reduction associated with manic episodes. The finding in Study 4 was further investigated in **Study 5** where we found that a further volume reduction occurred in patients that had manic episodes between baseline and follow-up but not in those that did not suffer from a manic episode.

The aim of these studies was to analyze gray matter volumes in the brains of people with bipolar disorder to investigate if there were differences between patients and healthy controls and if there were differences within the patient group. We have shown that the patient group has smaller gray matter volume in the bilateral insula and the medial prefrontal cortex. These findings have also been shown in other studies which indicate that this difference is highly probable (Selvaraj et al., 2012). We have also shown that other cortical areas are reduced in volume in bipolar disorder but that these alterations are associated with symptoms and illness progression rather than the illness per se.

Before drawing any conclusions, I would like to return to the diagnostic criteria for bipolar disorder. The individuals who have been examined in this thesis have – strictly speaking - only a few things in common; they have either had an episode of elevated mood for more than one week, or one episode of slightly elevated mood for four days and an episode of depression for two weeks. An important question to bear in mind when interpreting the results is: Do all these people have the same illness and does this illness have an underlying biology that is the same for all cases?

### 5.1 METHODOLOGICAL CONSIDERATIONS?

First, our findings underline the importance of thorough phenotyping of participants in MRI-studies of bipolar disorder, including an assessment of the medical treatment history. A group of severely ill patients with a history of psychoses and many illness episodes may show widespread cortical volume reduction when compared with healthy controls, but not all of these differences are necessarily related to the bipolar disorder per se. It is therefore important to have a large sample size that enables control for confounding factors. But this is difficult to ascertain given the cost and time necessary to conduct large scale MRI studies. Alternatively,

the sample should be homogeneous, but narrow inclusion criteria risk reducing the generalizability.

Second, the results of these studies can increase knowledge of the biological underpinnings of bipolar disorder. A particular facet when studying bipolar disorder is, however, that the illness is chronic but the patients are not chronically ill. The studies in this thesis were performed on euthymic patients. This means that none of the findings are associated with the symptoms of acute mania or depression. It is more likely that the volume reductions found in study I, II, and III represent a vulnerability to develop mania, hypomania, depression, or psychosis. We know that bipolar disorder is not a single-gene disease and research has not presented any single environmental factor that causes the illness. Therefore it is likely that bipolar disorder have multifactorial causes. The findings from Study III suggest that psychotic symptoms during mood episodes in bipolar patients are associated with a smaller cortical volume in the DLPFC and fusiform gyrus. Yet, these persons are not chronically psychotic. The psychosis requires some other factor, in this case mania or depression, to develop. Analogous, persons with smaller volume in the insula and mPFC are more likely to develop mania, or depression but the acute symptoms require some other triggering factor.

## **5.2 CAN THE RESULTS FROM THESE STUDIES GUIDE DIAGNOSTIC DECISIONS?**

A relevant clinical question is whether the findings in this thesis can guide diagnostic assessments. Thus, can a brain MRI-scan inform the clinician about whether a patient has bipolar disorder or some other psychiatric disorder? Our studies were, however, not designed to answer that question. All cases were diagnosed with bipolar disorder prior to the brain scan, and all controls were known not to suffer from a psychiatric disorder prior to inclusion. Hence, we cannot apply the results we get from analyses between bipolar disorder and healthy controls to inform clinical diagnostic decisions. Moreover, the methodology we have used in these studies did not have the specificity to distinguish a person with bipolar disorder from a healthy person on an individual level. The effect sizes of the volume reductions found in the bipolar vs. controls whole-brain study represents a Cohen's  $d$  of  $\sim 0.6$  which means that approximately  $\frac{3}{4}$  of the subjects will overlap in insular volume. More importantly, the clinical challenge is not to differentiate bipolar disorder from a healthy person: When patients present to psychiatric care, we know from the outset that the person have some psychiatric illness. It can be more challenging to distinguish a first manic episode with psychosis from a first-episode schizophrenia, and even more challenging to separate bipolar II disorder from borderline personality disorder, ADHD, or recurrent depression. We therefore need studies directly comparing cases with these different diagnoses. If such studies would reveal for example that the insular or medial prefrontal volume, or a combination of those, is a distinguishing trait for bipolar disorder, imaging techniques can be optimized for these regions.



### 5.3 SHOULD WE BELIEVE THE RESULTS?

When performing whole-brain analyses of gray matter volume, differences between groups can appear in unexpected regions of the brain. These differences can be true or caused by the various confounders listed in the introduction of this thesis. Analyses of brain morphology that are not designed to verify any other hypothesis than “There is probably some difference” must therefore be interpreted with precaution. Studies 1 and 2 showed structural differences between people with bipolar disorder and healthy controls in the insular and medial prefrontal cortices. Could a malfunction of these regions play a role in mania or depression? The insula monitors the state of the body by receiving input from various parts of the body regarding, e.g., heart rate, body temperature, and bowel movement. It summarizes these different factors into an emotional state. Through its connections with the medial prefrontal cortex, it creates a conscious feeling. The emotional state always precedes the feeling. The mPFC integrates the emotional state with context and past experience to create the feeling. For example, a marathon runner who has crossed the finishing line can feel happiness despite severe muscle pain, fast heart rate, high body temperature, and dehydration, i.e. visceral stimuli that normally are associated with discomfort or illness. But a malfunctioning mPFC might not be able to put the emotional state into context. In depression, the core symptom is that happiness cannot be felt in a context that past experience associates with this particular feeling. This can be due to a malfunction in the mPFC or that the emotional state is so severe that it cannot be modified by mPFC top-down regulation. In mania, the inhibitory regulation of behavior, based on past experience, is often insufficient, which leads to risk-taking and excessive involvement in pleasurable activities. An interesting discovery in this context is the involvement of the insula in the rare cases of ecstatic epileptic seizures (Gschwind & Picard, 2016). The authors describe cases of epilepsy that have ecstatic auras where the patient experiences enhanced physical well-being, heightened self-awareness and/or perception of the external world, feelings of overload, but in some cases also anxiety. These seizures can be elicited by electrodes planted in the anterior insula. The symptoms closely resemble the emotional state of mania apart from that their duration is much shorter. The biological causes of a true manic episode are unknown. The diversity in clinical presentation of mania might be explained by that an elevated emotional state, created in the anterior insula, is regulated differently by the mPFC, creating feelings of euphoria or irritability in different individuals and at different timepoints.

Based on what we know about the functions of insula and the mPFC, it is highly probable that these regions are involved in the development of the symptoms of both mania and depression.

The results of study 3 have been briefly discussed in the previous section. All three regions of GMV reduction have been associated with psychosis in other studies. Interestingly, the results lend support for the two-factor theory of psychotic symptoms (Coltheart, 2010). This theory implies that, for a delusion or hallucination to develop, a misinterpreted stimulus in

one brain region is not rejected as false in another region. Reduced volumes in the fusiform gyrus and the inferior frontal gyrus have been associated with hallucinations and may be the first factor of the model. The second factor would then be the rostral DLPFC, which is associated with reality testing.

The results from study 4, showing that reduced GMV in the bilateral DLPFC correlated to the lifetime number of manic episodes, are also in line with other studies of bipolar disorder. Smaller volume in the DLPFC, compared with healthy controls, has been associated with bipolar disorder, for example study 2. The most valid support of this result is our own results from study 5. The DLPFC is involved in executive functions but although some studies have shown a correlation between GMV in this region and cognitive performance (Hartberg et al., 2011) others have not (Alonso-Lana et al., 2016). DLPFC volume reduction has been found in patients with depression that do not reach complete remission (Li et al., 2010). Hence, GMV reduction in DLPFC is found in bipolar disorder. This reduction is, at least in part, progressive, but the correlation between this reduction and clinical presentation has yet to be established.

In summary, despite the exploratory designs of studies 1-4 the results of volumetric alterations have a high probability of being true. This is mainly due to the correction for confounders in the analyses, the stringent threshold for statistical significance, and last but not least the support gained from similar findings in other studies.

#### **5.4 IS BIPOLAR DISORDER A PROGRESSIVE NEURODEGENERATIVE ILLNESS?**

Little is known about premorbid gray matter volume alterations in patients with bipolar disorder. There are a few MRI studies performed on bipolar children and adolescents. Moreover, when interpreting such studies one must remember that these patients represent a subgroup of bipolar patients with a poorer outcome (Suominen et al., 2007) and the results cannot readily be generalized to the whole bipolar disorder group. Retrospectively, many adult patients have had symptoms of the illness in their teenage years but only the most severe cases get a diagnosis of bipolar disorder in that early age. A recent study of adolescents with bipolar disorder has shown that the gray matter volume in the anterior insula and DLPFC reduces over two years' time in the late teenage years (Najt et al., 2016). Our Study 5 focused only on the DLPFC but found no volume reduction in those patients who did not have any manic episodes during the seven-year follow-up. Studies of cognitive function have not established faster decline with ageing in bipolar patients than controls, but a negative association with the number of mood episodes, hospitalizations, and illness duration (Cardoso, Bauer, Meyer, Kapczinski, & Soares, 2015). Taken together, these findings suggest that bipolar disorder may be associated with gray matter volume reduction at the time of onset but further reductions depend on the course of the illness. This means that there can be

different causes to the volume reductions found at onset and those developing during the course of illness. Chronic stress causing high cortisol levels and glutamate-induced excitotoxicity has been suggested as a mechanism for progressive volumetric changes (Olney, Newcomer, & Farber, 1999) (Czeh et al., 2007). Classical biomarkers of neurodegeneration are not found in bipolar disorder ( $\beta$ -amyloid, gliosis, Lewy body inclusions) (Jakobsson et al., 2013). A recent review of the literature (Savitz, Price, & Drevets, 2014a) defines bipolar disorder as a “progressive neurodevelopmental disorder”. That means that the causes for the onset of the illness are neurodevelopmental but that the manic and depressive episodes cause glial and neuronal degeneration. This definition of the illness fits well with the results in this thesis since we find volume differences in disparate areas when comparing patients and healthy controls from when analyzing differences correlated to illness progression in the patient group.

## **5.5 WHAT IS REDUCED?**

The differences in gray matter volume between healthy controls and patients with bipolar disorder are located in the insula and medial prefrontal cortices. The fronto-insular region has a relatively high density of von Economo neurons (VENs). Studies of behavioral variant fronto-temporal dementia, displaying mania-like symptoms, have shown volume reduction and degeneration of these von Economo neurons in the fronto-insular region (Seeley et al., 2007; Seeley, Crawford, Zhou, Miller, & Greicius, 2009). Also, a study of persons with alcoholism showed a 10% smaller anterior insular volume and 60% less von Economo neurons post mortem (Senatorov et al., 2015). Interestingly, there are more von Economo neurons in the right hemisphere. The right/left ratio is 1.24 in the anterior insula and 1.9 in the ACC in the adult brain (Allman et al., 2010). It was on the right side that we in Study 1, as well as a meta-analysis (Selvaraj et al., 2012), found the largest difference in volume.

The von Economo neurons are much larger than pyramidal cells and their apical and basal dendrites are symmetrical. The symmetry suggests that the neuron compares input from both ends (Watson, Jones, & Allman, 2006). The dendritic branching is narrow and the VEN is believed to rapidly relay output from one very specific area to another. The VENs of the anterior insula and ACC are activated by social errors. During a manic state, the reactions to one's own social errors are often markedly reduced but the reactivity and awareness of others' is increased. There are no studies of insular VEN density in bipolar disorder, but one study has shown normal VEN density in the ACC (Brune et al., 2010). If the VEN density is not reduced in BD there may still be other implications that the VENs are affecting gray matter volume. Single nucleotide polymorphisms of the DISC1 (disrupted in schizophrenia 1) gene have been associated with bipolar disorder. 90-94% of VENs in humans expresses the protein encoded by DISC1 compared to 34-37% of other layer 5 pyramidal neurons (Allman et al., 2010). The DISC1 protein regulates the dendritic branching of neurons (Duan et al., 2007) and one single nucleotide polymorphism (SNP) of the DISC1 gene is associated with

reduced ACC volume (Hashimoto et al., 2006). The volume reduction can be caused by altered dendritic branching rather than reduced neuronal density.

Post-mortem studies have shown reduced glial density in the medial prefrontal cortex (Ongur et al., 1998) as well as in the DLPFC (Rajkowska et al., 2001). Both these areas have been shown reduced in bipolar disorder, although our study 1 found less volume only in mPFC. The role of glia cells in bipolar disorder is an emerging research field. Most studies have focused on oligodendrocytes. These cells represent 35% of all glia cells in the brain and one of their functions is to provide myelination of neurons. In the healthy brain, myelination continues until the age 40 but there are data suggesting reduced age-related cortical myelination in bipolar disorder (Vostrikov & Uranova, 2011). Oligodendrocytes express AMPA glutamate receptors and are therefore sensitive to damage caused by excess glutamate-mediated activation (McDonald, Althomsons, Hyrc, Choi, & Goldberg, 1998). In study 5, we showed that patients that had manic episodes decreased in cortical volume in the DLPFC. This could be due to stress-induced oligodendrocytal damage. Also, the levels of cortisol and other stress hormones are increased both during depression and mania. Chronic administration of cortisol to rats has shown to induce reorganization of the dendrites of pyramidal neurons in the DLPFC (Radley et al., 2004). Patients with no episode showed no volume change but an increase in cortical thickness. Atypical antipsychotic drugs are thought to promote intracortical myelination (Bartzokis et al., 2012). Many studies measuring biomarkers in CSF have found markers of microglial activation associated with all stages of bipolar disorder. A link between dysfunctional emotional regulation and low glial cell count has though not been established.

## **5.6 FREESURFER OR VBM, WHICH ONE IS THE PREFERABLE METHOD?**

In this thesis, we used two different softwares for analyzing brain structure, Statistical Parametric Mapping and Freesurfer. Both methods uses differences in T1 signal intensity to segment images into different tissue classes (GM, WM, CSF), but the method of segmentation differs. A few multimodal studies have found differences in cortical volume measures between Freesurfer and the SPM8 version (Popescu et al., 2016; Rajagopalan & Pioro, 2015). The differences are though not the same across studies. In one study, the Freesurfer method showed more positive findings than SPM and vice versa in the other.

When interpreting studies of brain volumetry, no matter what software, it is important to recognize what method of correction for multiple comparisons that has been applied and what level of statistical significance that has been used.

## **5.7 HOW SHOULD FUTURE STUDIES OF BRAIN STRUCTURE BE CONDUCTED?**

MRI-studies of humans are time consuming and expensive. As we have seen in the studies in this thesis there are a lot of factors that confound the results of volumetric analyses of the brain. Some of them are strictly methodological and can quite feasibly be dealt with. For example using the same scanner and scanning parameters, scanning subjects at the same time of day. Medications such as lithium alter the T1-signal but tampering with the medication conveys a substantial risk for the patient and should be avoided. Selection of subjects is of course dependent on the hypothesis. When it comes to studies of bipolar disorder it is often hard to choose exclusion criteria. If one narrows the criteria only to include bipolar I patients with no comorbidities one is not guaranteed to find results that are generalizable to the whole bipolar population. Since 67% of bipolar I patients have experienced some other psychiatric illness these “pure” cases should rather be considered as a subgroup. Analyses of large cohorts of patients that are phenotyped in detail can describe volumetric changes associated with each comorbid condition.

Multi-center studies are effective in collecting a large number of patients in a shorter time and the costs are spread over many institutions. To this day, only smaller prospective multi-center studies have been conducted. Data in the present neuroimaging consortia is collected from studies already performed at the collaborating centers. This means that differences in MRI-scanner settings will confound the results. Even though the total number of subjects is large in these studies each center must provide a fairly large number of subjects and healthy controls to enable analyses of group x center interactions. As we have seen, the effect sizes in whole brain analyses of bipolar disorder are small, partly because of the correction for multiple comparisons. Therefore, present studies have mostly focused on subcortical structures that can be automatically segmented.

No matter how well conducted the studies are, the main question is if structural imaging studies can solve the mystery that is Bipolar Disorder. We do not know the answer to that question yet. To come closer to an answer, studies of brain structure in the different polarities of the illness must be performed. Almost all studies have included euthymic patients. If we were to find differences in brain structure in the same individual that varies during mania, euthymia, and depression, then the importance of structural studies would increase. Also, we do not yet know what image resolution the next generations of imaging tools will provide. Where we stand today, structural imaging cannot find the answer to what causes bipolar disorder, but the results presented in this thesis can guide studies of other modalities, such as histopathological analyses. Since we have robust findings of decreased cortical volume in a few regions, future studies should focus on what type of cells that are reduced in volume or number in these regions.



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